Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article https://doi.org/10.1016/j.apjtb.2017.09.016

Antifouling evaluation of extracts from Red Sea soft corals against primary biofilm and biofouling

Yosry Abdel Aziz Soliman[1], Ahmed Mohammed Brahim[1*], Ahmed Hussein Moustafa[2], Mohamed Abdel Fattah Hamed[1]

[1]*Marine Chemistry Lab., Marine Environment Division, National Institute of Oceanography and Fisheries, Suez, Egypt*

[2]*Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt*

ARTICLE INFO

Article history:
Received 21 May 2017
Received in revised form 9 Sep 2017
Accepted 20 Sep 2017
Available online xxx

Keywords:
Antibacterial
Antifouling
Paints
Seawater
Marine
Organisms

ABSTRACT

Objectives: To evaluate antifouling property of extracts from Red Sea soft corals against primary biofilm and biofouling.

Methods: Seven species of soft corals *Sarcophyton glaucum* (a), *Sinularia compressa*, *Sinularia cruciata* (a), *Heteroxenia fuscescens* (a), *Sarcophyton glaucum* (b), *Heteroxenia fuscescens* (b) and *Sinularia cruciata* (b) were chosen to test their extracts as antibacterial and antifouling agents in Eastern Harbour of Alexandria, Mediterranean Sea. Bioactive compounds of soft corals were extracted by using methanol and concentrated under vacuum. The residues of extracts were mixed in formulation of inert paint which consisted of rosin, chlorinated rubber and ferrous oxide against micro and macro fouling organisms. The formulated paints were then applied on PVC panels twice by brush, hanged in a steel frame and immersed in Eastern Harbour of Alexandria Mediterranean Sea followed by visual inspection and photographic recordings.

Results: After 185 days of immersion in seawater, the antifouling results agreed with the antibacterial results where extracts of *Sinularia compressa* and *Heteroxenia fuscescens* (b) gave the best activity against marine fouling tubeworms and barnacles. The inhibition activity was correlated with the major functional groups (hydroxyl, amino, carbonyl, aliphatic (fatty acids), C═C of alkene or aromatic rings and C−Cl of aryl halides) of the extracts.

Conclusions: The strong antifouling activity makes them promising candidates for new antifouling additives. After the screening and application of natural organic compounds from soft corals, marine organisms show activity against micro and macro fouling organisms.

1. Introduction

Marine bio-fouling can be defined as the growth of unwanted organisms on the surface of artificial structures immersed in water [1,2]. Bio-fouling causes huge material and economic costs of maintenance of marine structures, naval vessels, and seawater pipelines [1]. It is estimated that governments and industry spend over $6.5 billion annually to prevent and control marine bio-fouling [3]. Further, ecological implications of bio-fouling include increased carbon emission and potential dispersion of invasive alien species [4–6]. Antifouling is the process of controlling or mitigating the settlement of fouling organisms on a surface. Commercial antifouling techniques include mechanical cleaning, biocides, toxic antifouling coatings and foul release or easy clean coatings. Amongst the above, antifouling paints containing toxic chemicals are the main strategies used against bio-fouling in the past. Tri-butyl tin was the most effective component in antifouling paints which was detrimental, not readily degraded in the natural environments and had non-targeted toxicity on organisms [7]. This property has led the International Maritime Organization to prohibit its application to ships since 17 September 2008 [8].

*Corresponding author: Ahmed Mohammed Brahim, Marine Chemistry Lab., Marine Environment Division, National Institute of Oceanography and Fisheries, Suez, Egypt.

E-mails: ahmed_pending@yahoo.com, ah_hu_mostafa@yahoo.com (A.M. Brahim).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Please cite this article in press as: Soliman YAA, et al., Antifouling evaluation of extracts from Red Sea soft corals against primary biofilm and biofouling, Asian Pac J Trop Biomed (2017), https://doi.org/10.1016/j.apjtb.2017.09.016

The substitutes of tributyl tin, such as Irgarol 1051 and Diuron, have also been found to be harmful to many non-targeted organisms [7,9]. Hence, alternative and environmentally acceptable, safe and effective antifouling substances are needed for incorporation into antifouling coatings, and these may include natural products isolated from certain marine organisms [10]. Incorporation of natural repellent products into antifouling paints has been tried by some researchers [11,12]. For this, a wide range of marine natural products have been screened for their activity concerning antimicrobial, antifungal, antialgal and antilarval properties [10,13,14]. Compounds with antifouling potential have been studied intensively in various marine sponges [15,16] and algae [17–19]. Marine natural products or crude extracts with antifouling activity have been reported from many marine organisms including marine bacteria, seaweeds, sea grasses, bryozoans, ascidians, cnidarians and sponges [10,20,21]. Antifouling and biological activities of marine macrophytes have been extensively studied by many researchers in various species of mangroves [6] seaweeds [22] and sea grasses [23,24]. In continuation to the previous study, this work depended on the extraction of natural products of soft corals [25] from Hurghada and Sharm El Sheikh to evaluate the bioactive and antifouling. The active functional group of the extracted organic compounds against biofouling was detected by using infrared spectroscopy.

2. Materials and methods

2.1. Sample collection, identification and extract preparation

The soft corals were collected by using SCUBA diving at different depths from Hurghada and Sharm El Sheikh, Egypt, on the Red Sea, in November 2013 and April 2014, respectively. Then, the collected samples were kept at −20 °C at the National Institute of Oceanography and Fisheries, Suez branch. Red Sea soft corals from Hurghada were identified as *Sarcophyton glaucum* (*S. glaucum*) (a) 1.5 m, *Sinularia compressa* (*S. compressa*) 2 m, *Sinularia cruciata* (*S. cruciata*) (a) 1.5 m and *Heteroxenia fuscescens* (*H. fuscescens*) (a) 2 m and the corals from Sharm El Sheikh as *S. glaucum* (b), 0.5 m, *H. fuscescens* (b), 10 m and *S. cruciata* (b), 10 m. The extraction processes of the bioactive organic compounds were as follows: About 1200 g of *S. glaucum* (a), 411.4 g of *S. compressa*, 713.77 g of *S. cruciata* (a), 329.27 g of *H. fuscescens* (a), 408.907 g of *S. glaucum* (b), 413.762 g of *H. fuscescens* (b) and 284.683 g of *S. cruciata* (b) were prepared. After cleaning and cutting into small pieces, methyl alcohol was used to extract the bioactive compounds three times for 10 d. The extract was concentrated under vacuum, the residue was washed three times by using ethanol to eliminate the inorganic salts, and then the filtrate was evaporated under vacuum to afford the bioactive organic compounds as crude.

2.2. Bacterial characterization

A microtitre assay by Andrews [26] was performed to determine the minimal inhibitory concentration (MIC) of the crude extract on different bacterial isolates. All extracts were diluted with dimethyl sulfoxide to prepare stock solutions of 100 mg/mL. A serial dilution of each stock solution was then performed into sterile nutrient broth. Different concentrations from each pure extract were made (100, 50, 25, 10, 5, 1, 0.5, and 0.1 mg/mL). For each concentration in nutrient broth 75 μL was pipetted into horizontal wells of well cell culture plate (1 well per concentration per bacterial isolate). The above procedure was repeated for each extract and the combinations, giving final well concentrations. Each plate was incubated in incubator at 32 °C for 24 h. Following incubation wells were observed for turbidity. MICs were taken as the lowest concentrations not showing any visible growth. Minimum bactericidal concentration (MBC) was also determined by removing 2 μL volume of the medium from each microtitre plate well and spotting onto sensitive agar. Agar plates were incubated for 18 h at 30 °C. Any growth observed from the spots was designated as an ineffective bactericidal concentration of extracts [27].

The well-cut diffusion technique was used to test the ability of different concentrations from the pure extract to inhibit the growth of indicator bacteria. About 50 mm of seawater agar medium inoculated with indicator microorganism was pored after solidification into plates. Wells were punched out by using 0.5 cm cork borer, and each of their bottoms was then sealed with two drops of sterile water agar. One hundred micro-liters of tested extracts were transferred into each well. All plates were incubated at 30 °C for 24 h; the detection of clear inhibition zone around the wells was an indication of antimicrobial activities of the different isolates.

2.3. Paint preparation, panel and frame preparation and field anti-macrofouling assays

The extracts were incorporated into inert matrix ingredients which consisted of 40 g rosin, 20 g chlorinated rubber, 10 g ferrous oxide, 20 mL dioctyl phthalate and 40 mL xylene in porcelain bottle jar (1 L) containing porcelain balls for stirring the components to form homogeneous paint. The formulated paints applied on PVC panels by brush were immersed in seawater of Eastern Harbour of Alexandria to investigate their antifouling profile under harsh marine conditions such as microorganisms in hydrothermal vents, corals …*etc*. Seven coating paint formulations (AF_1, AF_2, AF_3, AF_4, AF_5, AF_6 and AF_7) have been prepared by incorporating the extracts of *S. glaucum* (a), *S. compressa, S. cruciata* (a), *H. fuscescens* (a), *S. glaucum* (b), *H. fuscescens* (b) and *S. cruciata* (b) (2 g of tested extract/48 g of paint), respectively. In addition, the inert paint formulation was used as a control.

A 0.2 cm thick sheet of PVC panel was cut into 10 cm × 15 cm × 0.2 cm panels which were roughened by using emery papers at different grades from a coarse one to finer one. These panels were coated from both sides with two successive coats of the formulated paint. The paints were prepared by blending definite amounts of binder, pigments, plasticizer, then extracted compounds and solvents in a high-speed centrifuging ball mill, and were allowed to dry for 2 d between each coating. The coated panels were connected to the testing iron frames with nylon threads through nails bored in the panels.

All panels were immersed in the Eastern Harbour of Alexandria at a depth of 1.5 m, where the antifouling performance of each coated panel was studied periodically from 5 May 2015 to 10 November 2015 by visual inspection and photographic recordings. After a definite time, the panels were taken out of the sea, carefully washed with seawater and photographed. Then, they were immediately placed into the seawater to continue the

Please cite this article in press as: Soliman YAA, et al., Antifouling evaluation of extracts from Red Sea soft corals against primary biofilm and biofouling, Asian Pac J Trop Biomed (2017), https://doi.org/10.1016/j.apjtb.2017.09.016